

Claims

1. On-line detection method comprising the steps of: contacting an effluent of a fractionation step with a controlled amount of an enzyme; allowing the enzyme to interact with analytes suspected to be present in the effluent; addition of a controlled amount of a substrate for said enzyme; allowing a reaction of the enzyme with the substrate providing one or more modified substrate products; and detection of unreacted substrate, or a modified substrate product using a mass spectrometer.  
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2. The method according to claim 1, involving detection of a modified substrate product.
- 10 3. The method according to claim 2, wherein the reaction mixture passes a hollow-fibre module, separating off molecules which have a higher molecular weight said modified substrate product, prior to entering the mass spectrometer.
- 15 4. The method according to any one of the preceding claims, wherein the detection is based on specific m/z values for the substrate or the modified substrate product.
5. The method according to any one of the preceding claims, using electrospray ionisation mass spectrometry.
- 20 6. The method according to any one of the preceding claims, wherein a make-up flow is added to the reaction mixture resulting from the reaction with the substrate, prior to the introduction in the mass spectrometer.
7. The method according to any one of the preceding claims, wherein said fractionation step is a liquid chromatography separation, a capillary electrophoresis step or a combinatorial chemistry system.
- 25 8. The method according to any one of the preceding claims, wherein the liquid chromatography separation step is an HPLC, a reversed phase HPLC, a CE, a CEC, an IEF or an MEKC step.

9. The method according to any one of the preceding claims, using a mass spectrometer selected from the group consisting of electrospray ionization type, atmospheric pressure ionization type, quadrupole type, magnetic sector type, time-of-flight type, MS/MS, MS<sup>n</sup>, FTMS type, ion trap type and combinations thereof.
10. The method according to any one of the preceding claims, wherein said enzyme is a mixture of two or more different types of enzymes and said substrate is present in a mixture of different substrates, each of which substrate being specific for one of said enzymes.
- 10 11. The method according to any one of the preceding claims, wherein the enzyme or one of the enzymes is a protein kinase, and wherein the detection is carried out on a phosphorylated product of a kinase catalysed reaction.
12. On-line detection method, wherein a mass spectrometer with a multiple-inlet unit is used, to which multiple-inlet unit different fractionation lines are connected, wherein each fractionation line comprises an effluent to which controlled amounts of enzyme and known substrates are added as described in each of the previous claims.
13. Compound detected by the method of any one of the preceding claims.